THE INFLUENCE OF DESICCATION ON CERTAIN NORMAL IMMUNE BODIES

HOWARD T. KARSNER AND KATHERINE R. COLLINS

*From the Department of Pathology, School of Medicine, Western Reserve University, Cleveland, O.**

Many methods of drying have been applied to serum, vaccines and other substances for the purpose of preserving conveniently for a considerable length of time, under variations in temperature, the essential activities of such products. The practical objections to the results thus obtained are too great a loss of titer and the difficulty of solution.

Shakell[1] claims to have overcome these objections to a certain degree. By freezing liver and guinea-pig serum, while desiccation was going on, over sulphuric acid in vacuo, hydrolysis was prevented, the diastatic ferment of the liver and the complement of the serum were preserved. On dissolving the dried serum a clear solution resulted. Harris[2] applying this method to rabic brains, demonstrated activity after 4 months, the virus retaining from ⅓ to ½ its original virulence. Van Steenberge[3] was able to maintain virulence for several months by spreading brain emulsion in thin layers on porcelain plates and desiccating very rapidly in vacuo. Marie[4] repeated these experiments with some success. Shakell and Harris[5] believe that the thinly spread material is frozen by the rapid evacuation, that freezing maintains the particles in their normal relations so that the concentration of salts and other substances, which at ordinary temperature are in solution, is prevented. Drying, they believe, proceeds from the surface from cell to cell.

In our opinion this explanation does not seem adequate. It is quite possible that the spreading and rapid drying facilitates the cell to cell drying, while the cold may play an entirely different rôle by checking processes of a fermentative nature. In our experiments, serum dried in the upper part of the desiccator which was not immersed in the freezing mixture, but evacuated under the same conditions, showed a lower agglutinating index than the serum that was frozen.

Achalme's[6] work with smallpox vaccine helps to bear out this point. He states that the action of glycerol is not directly bactericidal, but by extracting the soluble ferments from the cells sets free an agent which is the destructive element. He maintains further that glycerol added to the crude material acts better than when added to the finely triturated substance, as in the latter case the ferments are set free more completely, and being in excess attack not only the bacteria, but the vaccinal agents as well.

Burrows and Cohn,[7] in a quantitative study of the evaporation of blood serum, found that prolonged warming attendant on drying in a current of air

Received for publication July 21, 1919.

* Supported by a special grant from Mr. H. G. Dalton.

[1] Jour. Physiol., 1909, 24, 325.
[2] Jour. Am. Pub. Health Assn., 1911, 7, 52.
[3] Compt. rend. Soc. de Biol., 1903, 55, 1046.
[4] Quoted by Shakell and Harris, 5, 1.
[5] Shakell and Harris, Jour. Infect. Dis., 1911, 8, 47.
[6] Bull. de la Soc. de Path. exotique, 1909, 2, 431.
[7] Jour. Biol. Chem., 1918, 36, 587.

at atmospheric pressure tended to denature the proteins. They resorted to drying under reduced pressure in the presence of calcium chlorid at a temperature between 45 and 50 C. They observed that the best results were obtained if the serum was allowed to drop slowly on the sides of the flask so timed as to make the rate of evaporation and the addition of fresh serum correspond. They claim that serum so dried is practically free from electrolytes, and is faintly alkaline, the P_H being 8.1, while the original serum was 7.6. Redissolved in its original concentration the reaction of the dried serum was P_H 0.4.

Most of the work reported in connection with immune serum has been with serum of high titer, in which even fairly high percentage losses of titer would still leave the serum with satisfactory properties. Little work has been done with serum containing normal immune bodies, usually of low titer, in which considerable percentage losses would make a great difference in their practical use. The following experiments were conducted for the purpose of making a comparative study of the effects of the different methods of drying on the normal antibodies in the blood of different animals.

It was found that the blood of the normal horse and normal goat contained agglutinins for B. dysenteriae, Flexner type, and hemagglutinins for rabbit cells in sufficient quantity for a practical working basis.

The preparations were made by spreading 0.3 c c of serum on slides or in flat-bottomed glass dishes. The following methods of drying were followed: drying in the air or in vacuo over sulphuric acid at room temperature, and freezing and drying over sulphuric acid in vacuo. Normal horse serum was also dialyzed and treated in the same manner. Strips of filter paper were saturated with definite amounts of serum and dried in the air at room temperature. This latter method was not satisfactory on account of the excess amount of salt solution required to dissolve out the dried residue.

The dried serum was taken up by adding 0.85% salt solution up to the original amount, allowing to stand about 20 minutes, the solution and residue being thoroughly mixed and taken up. The slide was then washed off with the same amount of salt solution which was added to the first part. The clearness of the solutions differed irrespective of the method of drying employed, and did not appreciably affect the results. The frozen specimens on the whole, however, gave the clearer solutions. The freezing and desiccation over sulphuric acid in vacuo was carried out according to the method of Shakell, but it was not absolutely certain that in every instance the frozen state was maintained throughout the entire period of desiccation. The lower part of the desiccator was immersed in a freezing mixture and allowed to remain while the serum was being prepared. This cooled the dish and the air in the dish. The slides or dishes were placed on the floor of the desiccator which was turned rapidly about in the freezing mixture. A few moments sufficed to freeze the serum solidly; the sulphuric acid was then placed in the upper part of the desiccator, the cover properly adjusted and all connections sealed with a mixture of one part wax and six parts vaselin. Evacuation was produced to a pressure of from 15-40 mm. by means of a water pump. The freezing mixture was maintained at 10 C. for about 15 hours. The sulphuric acid was agitated from time to time to prevent accumulation of water on the surface. In two instances the desiccator was left in the freezing mixture for from 6 to 7 hours, then transferred to the freezing compartment of the refrigerator and allowed to remain for 3 days. There was not any apparent difference in the effects of the two methods.

The effects of desiccation on the agglutinins and hemagglutinins found in the serums of normal horse and normal goat and normal horse serum dialyzed are given in the following tables:

TABLE 1

NORMAL HORSE SERUM DRIED AND TREATED WITH B. DYSENTERIAE FLEXNER FOR AGGLUTININS

Number of Days Dried	Original Serum		Frozen and Dried over H_2SO_4 in Vacuo				Dried over H_2SO_4 in Vacuo				Dried in Air		
	1:400	1:500	1:50	1:100	1:200	1:300	1:50	1:100	1:200	1:300	1:100	1:200	1:300
11	+++	+	+++	+++	+++	+++	+++	+++	+++	++	+++	+++	—
20	+++	+	+++	+++	+++	+++	+++	+++	+++	—	+++	++	—
27	+++	+	+++	+++	+++	++	+++	+++	+	—	+++	+	—
41	+++	+	+++	+++	+++	+	+++	+++	—	—	omitted		
51	+++	+	+++	+++	++	—	+++	+++	—	—	omitted		
59	+++	+	+++	+++	++	—	+++	+++	—	—	+++	—	—
73	+++	+	+++	++	—	—	+++	+	—	—	omitted		
111	+++	+	+++	+	—	—	+++	+	—	—	omitted		

+++ complete agglutination; ++ fair agglutination; + trace of agglutination; — no agglutination.

TABLE 2

NORMAL HORSE SERUM DRIED AND TESTED WITH RABBIT CELLS FOR THE HEMAGGLUTININS

Number of Days Dried	Original Serum		Frozen and Dried over H_2SO_4 in Vacuo				Dried over H_2SO_4 in Vacuo				Dried in Air			
	1:12	1:16	1:4	1:8	1:12	1:16	1:4	1:8	1:12	1:16	1:4	1:8	1:12	1:16
13	+++	++	+++	+++	++	—	+++	++	—	—	+++	++	—	—
20	+++	++	+++	+++	—	—	+++	++	—	—	+++	++	—	—
51	+++	++	+++	++	—	—	+++	+	—	—	+++	+	—	—
59	+++	++	+++	++	—	—	+++	—	—	—	+++	—	—	—
73	+++	++	+++	—	—	—	omitted				+++	—	—	—
111	+++	++	omitted				++	—	—	—	omitted			

TABLE 3

NORMAL GOAT SERUM DRIED AND TESTED WITH B. DYSENTERIAE, FLEXNER, FOR AGGLUTININS

Number of Days Dried	Original Serum		Frozen and Dried over H_2SO_4 in Vacuo				Dried over H_2SO_4 in Vacuo				Dried jn Air			
	1:200	1:300	1:50	1:100	1:200	1:300	1:50	1:100	1:200	1:300	1:50	1:100	1:200	1:300
15	+++	—	+++	+++	+	—	+++	+++	—	—	omitted			
20	+++	—	+++	+++	—	—	+++	+++	—	—	+++	+++	—	—
25	+++	—	+++	+	—	—	omitted				omitted			
65	+++	—	+++	—	—	—	—	—	—	—	+	—	—	—
69	+++	—	+	—	—	—	omitted				omitted			

According to the tables, there is a decrease in the agglutinating power of the serum drying for 11 days both for B. dysenteriae and for rabbit cells. The serum that was frozen and dried over sulphuric acid in vacuo shows less loss than when dried over sulphuric acid in vacuo at room temperature, while that dried in air shows a still greater loss than the other two. The decline takes place a little more gradually with the frozen than with the nonfrozen serum, while the serum dried in the air loses its agglutinating power most rapidly of all. The ratio of loss for the various methods is fairly well maintained through the time.

Tables 3 and 4 give the results of drying normal goat serum.

TABLE 4

NORMAL GOAT SERUM DRIED AND TESTED FOR HEMAGGLUTININS WITH RABBIT CELLS

Number of Days Dried	Original Serum	Frozen and Dried over H_2SO_4 in Vacuo			Dried over H_2SO_4 in Vacuo			Dried in Air	
	1:12	1:4	1:8	1:12	1:4	1:8	1:12	1:4	1:8
15	+++	+++	+++	+	+++	+++	—	+++	—
20	+++	+++	+++	—	omit	ted		omit	ted
55	+++	+++	—	—	—	—		++	—
65	+++	+++	—	—	—	—		+	—
69	+++	+	—	—	—	—		omit	ted

TABLE 5

NORMAL HORSE SERUM DIALYZED AND TESTED WITH B. DYSENTERIAE FOR AGGLUTININS

Number of Days Dried	Original Serum		Frozen and Dried over H_2SO_4 in Vacuo			Dried over H_2SO_4 in Vacuo			Dried in Air			
	1:200	1:300	1:100	1:200	1:300	1:100	1:200	1:300	1:50	1:100	1:200	1:300
11	+++	—	+++	+++	—	+++	+++		+++	+++	+++	—
20	+++	—	+++	+++	—	+++	+++		+++	+++	+++	—
59	+++	—	+++	—	—	+++	—		+++	+++	—	—
67	+++	—	+++	—	—	—			+	—	—	—

TABLE 6

NORMAL HORSE SERUM DIALYZED AND TESTED WITH RABBIT CELLS FOR HEMAGGLUTININS

Number of Days Dried	Original Serum		Frozen and Dried over H_2SO_4 in Vacuo			Dried over H_2SO_4 in Vacuo		Dried in Air		
	1:12	1:16	1:4	1:8	1:12	1:8	1:12	1:4	1:8	1:12
15	+++	—	+++	+++	++	+++	++	+++	+++	+
25	+++	—	+++	+++	++	+++	++	+++	+++	—
55	+++	—	+++	+	—	omit ted		+++	—	—
65	+++	—	+++	—	—	omit ted		+	—	—

The findings are similar to those obtained with normal horse serum. Practically the same ratio of loss for all methods holds in the case of the goat serum as in the horse serum.

Tables 5 and 6 give the results of drying normal horse serum that had been dialyzed 4 days. After this length of time the serum failed to show a trace of sodium chlorid.

It will be noted that there was a loss of agglutinins during dialyzation. No loss occurred, however, after drying under 20 days, whereas the first loss in the nondialyzed serum occurred during the first 10 days. On the 59th day the bacterial agglutinins are uniformly reduced one-half; on the 67th day the strength of the frozen serum remains the same as on the 59th day, while the serums dried in the air and over sulphuric acid in vacuo have lost the agglutinating power in the dilution of 1:50. The hemagglutinins, with the exception of a slight loss over the original serum in the first 20 days, follow very much the same course.

The retention of the agglutinin content of the dried dialyzed serum lends support to the idea of Shakell and Harris that salt concentration brings about a loss in the active elements of various substances subjected to drying, but the subsequent decline of the agglutinins after a longer period of desiccation justifies our opinion that other factors also aid in the destruction of these properties.

Consideration, however, must be given to the possibility of nonedialyzable salts being present. Greenwald[8] found that serum dialyzed for 4 or 5 days still contained about 0.3 mg. of an acid soluble phosphorus per 100 c c.

We made some observations on the crystallization of serum. The results were sufficiently interesting to suggest future possibilities along these lines. As observed microscopically, the crystallization in the dried serum was uniform in all three methods but on dissolving in distilled water and recrystallizing by evaporation in air, a decided change took place in the formation of the crystals, certain forms being entirely absent. The dialyzed serum showed no definite crystall formation.

TABLE 7

NORMAL HORSE SERUM BACTERIAL AGGLUTININ INDEX 1:400. CELL AGGLUTININ INDEX 1:12

	Number Days Dried	Method of Drying	Bacterial Agglutinin Index	Hemagglutinin Index	PH
Normal horse serum dried	17	Frozen H_2SO_4 in vacuo.....	1:300	1:8	7.3
	17	Air.....	1:200	1:8	7.3
	51	Frozen H_2SO_4 in vacuo.....	1:200	1:8	7.2
	51	H_2SO_4 in vacuo.....	1:100	1:4	7.2
	56	Frozen H_2SO_4 in vacuo.....	1:200	1:8	7.2
	73	Air.....	1:100	0	7.3
	111	H_2SO_4 in vacuo.....	.....	4	6.6

NORMAL GOAT SERUM, BACTERIAL AGGLUTININ INDEX 1:200; CELL AGGLUTININ INDEX 1:12; PH 7.6

Normal goat serum dried	17	Frozen H_2SO_4 in vacuo.....	1:100	1:8	7.5
	65	Frozen H_2SO_4 in vacuo.....	1:50	1:4	6.6
	65	H_2SO_4 in vacuo.....	.....	...	...

The serum of two guinea-pigs was treated in the same manner as serum from the horse and goat and the complement content tested. One lot of serum with a complement content of 0.02 c c to the dose failed to act after 15 days of drying when two and a half times this amount was used. The second lot with an initial strength of 0.03 c c failed to act in the higher doses after 11 days. These results are contrary to those of Shakell and Harris with regard to complement.

In combination with an emulsion of B. dysenteriae, complement was completely fixed by 0.15 c c of the original normal horse serum. After 16 days drying it required five times this amount of the frozen serum to bind the complement. Six times this amount of serum dried over sulphuric acid in vacuo without freezing acted very feebly on the 20th day.

In some instances, the H-ion concentration was determined by the colorimetric method. The results are given in the following table together with the agglutinating index of the corresponding specimen of serum. The dried serum was dissolved in 0.85% salt solution and diluted ten times with distilled water. The distilled water and the salt solution were tested and were neutral.

According to these data the H-ion concentration of the dried serum undergoes a slight change which does not become marked until the agglutinins have reached a very low point.

Burrows found, as quoted, that by his method a decrease of P_H occurred in the dried serum over the original serum. He makes no statement, however, regarding the time allowed to elapse before the reactions were taken, but pre-

[8] Jour. Biol. Chem., 1916, 35, 431.

sumably they were taken on the completion of drying which should occur under 36 hours, while the above tests were after 17 days of drying.

Various phenomena that developed during the progress of the work indicate the possibility of many factors playing a part in the deterioration of immune bodies in the serum of the normal horse and goat, acting alone or interdependently.

The delay in the loss of agglutinins in the dialyzed serum points to salt concentration as one cause for this, either by effecting changes in surface tension of colloid particles or development of salt antagonism through interference with the law of direct proportions or variations in the electrolyte conditions. Application of the work of Osterhout[9] and of Loeb[10] on the subject of salt antagonism offers suggestions for further study of this phase of the problem. The delay of loss in frozen specimens as against the nonfrozen leads one to consider carefully the possibility of ferments being liberated and acting under the changed conditions. Another point that merits consideration is the relation of pseudoglobulins and euglobulins. The conversion of pseudoglobulins into euglobulins which plays such an important part in the concentration of antitoxin may find application to this problem of drying. Dean[11] thinks it quite possible that the pseudo- and euglobulins are different phases of physical aggregation in which case the many factors that may be acting during drying, especially salt concentration, might readily be the preceding cause.

SUMMARY

The normal antibodies in the serum of the horse and goat are gradually decreased by drying.

The agglutinins and hemagglutinins are less affected by drying if the serum is frozen while desiccation is going on.

The loss of titer in the nondialyzed serum takes place within the first 10 days while it is delayed in the dialyzed serum until after the 20th day.

The decrease of agglutinins in the dialyzed serum is uniform for all methods of drying until after 2 months, while in the nondialyzed serum the loss is greater in the serum that was not frozen and still greater in the serum dried in the air.

The P_H of dried serum is slightly greater than in the original serum and as the loss of agglutinins become greater the difference is more marked.

Dried serum dissolved and dried again shows definite changes in the formation of crystals.

It is probable that several factors rather than one alone are responsible for the changes in the antibodies produced by drying, these being of a physicochemical nature and acting interdependently.

[9] Jour. Biol. Chem., 1918, 363; Science, 1916, n. s. 44, 318.
[10] Jour. Biol. Chem., 1918, 34, 395; Science, 1912, 35, 112.
[11] Brit. Med. Jour., 1916, 2, 749.